Claim 34 (New): The method of Claim 21, wherein said observing further comprises measuring the cyclic adenosine monophosphate concentration.

REMARKS

1. Summary of Interview

A telephone interview on 7/16/03 was attended by Dr. Leonard Kohn, Mr. James Brown, Applicant's representative Dr. Maha Hamdan, and Examiner Nolan. At the interview, the pending claims and Applicant's proposed amendment that was sent via facsimile to the Office on 7/15/03 and 7/16/03 were discussed.

The Examiner indicated that the proposed amendments to Claims 19-21 overcome the outstanding rejection. Since the instant amendments to Claims 19-21 are the same as those in the above-referenced proposed amendments, Claims 19-21 are in **condition for allowance**.

The Examiner requested further evidence to overcome the rejection of Claims 1, 3-16, and 18 based on obviousness. Accordingly, Applicant submits further evidence as discussed below.

2. Status of the Application

Claims 1, 3-16, 18-21 are pending in the present application.

The amendments herein were made to describe particular embodiments of the invention, notwithstanding Applicant's belief that the unamended claims would have been allowable, without acquiescing to any of the Examiner's arguments, and without waiving the right to prosecute the unamended (or similar) claims in another application, but rather for the purpose of furthering Applicant's business goals and expediting the patent application process in a manner consistent with the PTO's Patent Business Goals (PBG).[1]

Briefly, Claims 1, 10 and 16 have been amended by changing "autoantibodies" to "antibodies" to provide antecedent basis for the term "antibodies" that appears in the originally-filed steps c) of Claims 1 and 10, and step d) of Claim 16.

Claims 1, 10, and 16 have also been amended to recite that the CHO-Rluc cells comprise a "reporter gene." Support for "reporter gene" is in the Specification, page 21, second and third paragraph, which says:

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[1] 65 Fed. Reg. 54603 (September 8, 2000).

> "The terms "reporter gene construct," or "reporter gene vector," as used herein refers to a recombinant DNA molecule containing a sequence encoding the product of a reporter gene and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. The term "reporter gene," refers to an oligonucleotide having a sequence encoding a gene product (typically an enzyme) which is easily and quantifiably assayed when a construct comprising the reporter gene sequence operably linked to a heterologous promoter and/or enhancer element is introduced into cells containing (or which can be made to contain) the factors necessary for the activation of the promoter and/or enhancer elements. Examples of reporter genes include but are not limited to bacterial genes encoding β-galactosidase (*lacZ*), the bacterial chloramphenicol acetyltransferase (*cat*) genes, firefly luciferase genes and genes encoding β-glucuronidase (*GUS*)."

Claims 1, 7, 8, 10, 13, and 14 have been amended by deleting the term "cultured" cells to underscore the fact that methods encompass cells that may be incubated in a starvation medium prior to treatment with PEG-containing medium, as taught in the Specification on page 31, lines 4-11; paragraph bridging pages 34 and 35; page 37, lines 24-25; page 40, lines 10-14; and page 41,lines 21-24.

Claims 1, 10, and 16 have been amended to recite that the reporter gene is "expressed," and observing "increased expression of said reporter gene in said cells in the presence of said test sample compared to in the absence of said test sample." Support is found in, for example, the Specification, Table 3 on page 38, which discloses increased expression of the exemplary luciferase reporter gene when the cells are treated with PEG and Graves' IgG compared to treatment with Graves' IgG in the absence of PEG.

Claim 4 has been amended to use similar language as Claim 18.

Claims 19-21 have been amended to change their form into independent claims by incorporating the limitations of the claims from which they depended, and by changing "said CHO-Rluc cells" to "a control sample comprising CHO-Rluc cells."

New Claims 22-28 are the same as Claims 3-9, except that they depend from Claim 19 instead of from Claim 1.

New Claims 29-33 are the same as Claims 11-15, except that the depend from Claim 20 instead of from Claim 10.

New Claim 34 is the same as Claim 18 except that it depends from Claim 21 instead of Claim 16.

Claims 1, 3-16, 18-21 have been rejected on the following grounds:

A. Claims 19-21 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of adequate written description, and

B. Claims 1, 3-16 nd 18 stand rejected under 35 U.S.C. §103(a) as being allegedly obviousness over Evans *et al.* in view of Yamashiro *et al.*

Applicant believes that the following remarks traverse the Examiner's rejection of the claims. These remarks are presented in the same order as they appear above.

A. Rejection of Claims 19-21 under 35 U.S.C. §112, first paragraph (written description)

Claims 19-21 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of adequate written description.[2] The Examiner argued that the artisan "would be **required** to perform an assay on said CHO-Rluc with bovine thyroid stimulating hormone on **the same cells** that were stimulated with thyroid stimulating autoantibodies as is **required** by claim 1," but the Specification discloses that "CHO-Rluc cells were **either** incubated with bovine TSH or thyroid stimulating autoantibodies, **not both**."[3]

Applicant has amended[4] each of Claims 19-21 by reciting "a control sample comprising CHO-Rluc cells" to reflect using bovine thyroid stimulating hormone and thyroid-stimulating antibodies in different samples.[5] Since this amendment found favor with the

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[2] Office Action, page 2, item 4.

[3] *Id.*

[4] The amendments herein were made to describe particular embodiments of the invention, notwithstanding Applicant's belief that the unamended claims would have been allowable, without acquiescing to any of the Examiner's arguments, and without waiving the right to prosecute the unamended (or similar) claims in another application, but rather for the purpose of furthering Applicant's business goals and expediting the patent application process in a manner consistent with the PTO's Patent Business Goals (PBG).

[5] Applicant notes that this amendment is not a limitation of the claims, but rather further describes a property of the CHO-Rluc cells. In other words, the claims as amended encompass incubating CHO-Rluc cells with one or more of thyroid-stimulating antibodies and bovine thyroid stimulating hormone. Thus, the claimed invention does not require (but may include) the use, in any order, of two CHO-Rluc samples: one

Examiner at the above-referenced telephone interview, Claims 19-21 are in **condition for allowance**.

New Claims 22-34 should also be allowed, since they depend from Claims 19-21, and are supported by Claims 3-9, 11-15, and 18.

B. Rejection Of Claims 1, 3-16 and 3-18 Under 35 U.S.C. §103 Over Evans *et al.* In View Of Yamashiro *et al.*

Claims 1, 3-16 and 18 continue to be rejected under 35 U.S.C. §103 for alleged obviousness over Evans *et al.*[6] in view of Yamashiro *et al.*[7] Applicant respectfully traverses.

The Examiner argued that "all of the components in the **generation of cAMP** in the Yamashiro *et al.*, assays and the Evans *et al.*, assay appear to be functionally identical"[8] and that "[w]hat is at issue is whether there was a reasonable expectation of success that PEG would **increase cAMP levels** in CHO-Rluc as it did porcine cells."[9]

This argument is incorrect. The issue is whether there was a motivation and reasonable expectation of success that treating CHO-Rluc cells with PEG would result in "**increased expression**" of the reporter gene, as recited by the claims. In this regard, it is important to note a difference between the prior art and the claimed invention is that the claimed methods recite treating the CHO-Rluc cells with "polyethylene glycol" (PEG) (compared to the absence of PEG in Evans *et al.*) and observing "expression" of the reporter gene in CHO-Rluc cells (rather than the generation of cAMP and/or measurement of cAMP levels as in Yamashiro *et al.*).

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sample treated with thyroid-stimulating antibodies, and the other sample treated with bovine thyroid stimulating hormone.

[6] Evans et al. (1999) "Development of a luminescent bioassay for thyroid stimulating antibodies," J. Clin. Endocrin. Metabolism 84(1)374-377.

[7] Yamashiro *et al.* (1999) "Mechanism of the Augmentative Effect of High Polyethylene Glycol (PEG) Concentrations on the Thyroid Stimulating Activity in TSAb-IgG Using a Porcine Thyroid Cell Assay," Endocrine Research 25:67-75.

[8] (Emphasis added) Office Action, page 4, item E. See, also page 3, items A and B, and page 4, items C and D.

[9] (Emphasis added) Office Action, page 5, first full paragraph.

There is no motivation or reasonable expectation of success in modifying the cited references because PEG's effect of **increasing** cAMP in Yamashiro *et al.*'s porcine thyroid cells that were treated with TSAb-IgG[10] cannot reasonably be extrapolated to **increasing** gene expression[11] in Evans *et al.*'s JP09 cells[12] that were treated with TSHR autoantibodies in the absence of PEG.[13] Indeed, the prior art **teaches away** from the claimed methods by showing that, rather than increasing gene expression, increased cAMP levels **decrease** expression of genes (such as TSH receptor and major histocompatibility complex class I) that are under the regulatory control of cAMP responsive elements (CREs). For example, Akamizu *et al.*[14] (Tab 1) states that with respect to the TSH receptor:

> "When IgG preparations from patients with Graves disease were tested, those which **increased cAMP** levels like TSH also **down-regulated TSH receptor mRNA levels** (Table 1, Graves IgG no. 1, representative of six tested)."[15]

Saji *et al.* confirmed this phenomenon in three subsequent publications (Tabs 2-4)[16] with

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[10] Yamashiro *et al.*, page 70, Figure 1.

[11] Evans *et al.*, page 376, Table 4.

[12] JP09 cells stably express the human TSHR and contain "2 cAMP response elements (CREs) in tandem, linked to the firefly luciferase gene." Evans *et al.*, page 374 paragraph bridging columns 1 and 2.

[13] Applicant also incorporates his prior arguments and evidence that were presented in the Responses mailed on 2/21/02 and 12/3/03 (including the Declaration by Dr. Leonard Kohn mailed on 12/3/03) with respect to the lack of motivation to combine the references, as well as a lack of reasonable expectation of success in practicing the claimed methods.

[14] Akamizu *et al.* "Cloning, chromosomal assignment, and regulation of the rat thyrotropin receptor: Expression of the gene is regulated by thyrotropin, agents that increase cAMP levels, and thyroid autoantibodies." Proc. Natl. Acad. Sci. USA, 87, 5677-5681 (1990).

[15] (Emphasis added) Akamizu *et al.*, page 5680, sentence bridging columns 1 and 2.

[16] Saji *et al.* "Increases in cytosolic Ca^{++} down regulate thyrotropin receptor gene expression by a mechanism different from the cAMP signal," Biochem. Biophys. Res. Commun., 176, 94-101 (1991) (Tab 2); Saji *et al.* "Regulation of thyrotropin receptor gene expression in rat FRTL-5 thyroid cells," Endocrinology, 130, 520-523, (1992 a) (Tab 3); Saji *et al.* "Hormonal regulation of major histocompatibility complex class I genes in rat thyroid FRTL-5 cells: Thyroid-stimulating hormone induces a cAMP-

regard to the major histocompatibility complex (MHC) class I. For example, Saji *et al.* (Tab 4) states that:

> "TSH binding to the TSHr is known to increase intracellular levels of both Ca^{2+} and cAMP ...**decreases in class I RNA levels** were observed after treatment of FRTL-5 cells with 8-Br-cAMP and with either forskolin or cholera toxin (Table 2), both of which cause **increased intracellular cAMP levels** ..."[17]

Importantly, Saji *et al.* (Tab 4) demonstrates that the effect of TSH (which is mediated via cAMP) on MHC class I gene expression is dependent on the TSH/cAMP concentration,[18] *i.e.*, higher levels of TSH/cAMP resulted in a greater **reduction** of gene expression than lower levels of TSH/cAMP.

Furthermore, Ikuyama *et al.* (Tabs 5 and 6)[19] used TSHR promoter-CAT plasmids in CAT reporter gene assays to show that:[20]

> "the promoter activity is specifically expressed in thyroid cells and is **negatively** regulated by TSH via its cAMP signal."[21]

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mediated decrease in class I expression," Proc. Natl. Acad. Sci. U.S.A., 89, 1944-1948 (1992 b) (Tab 4).

[17] Saji *et al.* (1992 b) (Tab 4), page 1946, first column, first paragraph, and Table 2.

[18] Saji *et al.* (1994 b) (Tab 4), page 1945, "Results" on columns 1 and 2; and Table 1.

[19] Ikuyama *et al.* "Characterization of the 5'-flanking region of the rat thyrotropin receptor gene," Mol. Endocrinol., 6, 793-804 (1992 a) (Tab 5); and Ikuyama *et al.* "Role of the cyclic adenosine 3',5'-monophosphate response element in efficient expression of the rat thyrotropin receptor promoter," Mol. Endocrinol., 6, 1701-1715, (1992 b) (Tab 6).

[20] See also, al. Shimura *et al.* "The cAMP response element in the rat thyrotropin receptor promoter," J. Biol. Chem., 268, 24125-24137 (1993) (Tab 7); and Shimura *et al.* "Thyroid-specific expression and cyclic adenosine 3',5'-monophosphate autoregulation of the thyrotropin receptor gene involves thyroid transcription factor-1," Mol. Endocrinol., 8, 1049-1069 (1994) (Tab 8), which subsequently defined the mechanism for this and showed it was complex, involving multiple promoter elements and trans factors other than CREB in CRE-containing promoter-luciferase constructs.

[21] (Emphasis added) Ikuyama et al (1992 a) (Tab 5), page 793, column 2, first full paragraph..

It is important to note that Saji *et al.*[22] (Tab 9) subsequently demonstrated in Figure 1 that heterologous gene expression in constructs with multiple CRE sites was even more **suppressed** by TSH/cAMP than in constructs with fewer CRE sites. Furthermore, in Figure 5, footprinting of the CRE establishes that TSH/cAMP does not increase CREB binding but rather **decreases** it.

The complexity of elements and CRE binding proteins that are involved in the TSH-cAMP **downregulation** of the MHC class I was further defined by Kirchner *et al.*[23] (Tab 10) who showed further complexity because of CRE binding inhibitory CREB analogs.

In sum, in view of the known complexity of transcriptional gene regulation in general as discussed in Brivanlou and Darnell[24] (Tab 11), and of the particular teachings of the prior art (Tabs 1- 10) that increased cAMP levels **decrease** the levels of expression of genes that are under the regulatory control of CREs, one of skill in the art would **not** be motivated to treat the invention's CHO-Rluc cells with PEG, since Yamashiro *et al.* shows that PEG increases cAMP levels. Furthermore, based on the prior art's teachings in Tabs 1-11, even if the artisan treated the invention's CHO-Rluc cells with PEG in parallel to Yamashiro *et al.*'s disclosure, the artisan would **expect** PEG to increase cAMP levels as taught by Yamashiro *et al.*, and thus to **decrease expression** of the reporter gene as shown by each of the references in Tabs 1-10. Since this expected result is the **opposite** of that recited in the claims, a reasonable expectation of success is lacking. This negates a *prima facie* case of obviousness.

In view of the above, it is respectfully requested that the rejection of the claims under 35 U.S.C. § 103 be withdrawn.

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[22] Saji *et al.* "Regulation of major histocompatibility complex class I gene expression in thyroid cells," J. Biol. Chem., 272, 20096-20107 (1997).

[23] Kirshner *et al.* "Major histocompatibility class I gene transcription in thyrocytes: a series of interacting regulatory DNA sequence elements mediate thyrotropin/cyclic adenosine 3',5'-monophosphate repression," Mol. Endocrinol., 14, 82-98 (2000).

[24] Brivanlou and Darnell, Jr., "Signal transduction and the control of gene expression," Science 295 813-818 2002, particularly pages 814-816.

CONCLUSION

All grounds of rejection of the Office Action of February 24, 2003 having been addressed, the claims are in condition for allowance. Applicant encourages the Examiner to **call the undersigned collect before beginning to draft a written communication**, if any.

Signed on behalf of:

Dated: July 24, 2003

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